We claim:

1. A modified pneumolysin polypeptide having attenuated hemolytic activity wherein said modified pneumolysin polypeptide is obtained by:

a) randomly mutating a nucleic acid molecule encoding for wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides and expressing the mutated nucleic acid molecules in host cells;

b) assaying the modified polypeptide expressed by the host cells for hemolytic activity;

c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

2. A modified properly-refolded pneumolysin polypeptide having attenuated hemolytic activity comprising an amino acid sequence of type 14 pneumolysin wherein at least one amino acid in the region comprising amino acid residues 1 to 257 is substituted and wherein at least one of said amino acid substitutions results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

. The modified pneumolysin polypeptide of claim 2, wherein the hemolytic activity is less than 25% compared to wild-type pneumolysin.

4. A modified pneumolysin polypeptide according to claim 3, comprising at least one amino acid substitution in the amino acid sequence of Formula I at residue positions 61, 148, or 195 or the combination of substitutions at residue positions 33, 46, 83, 239 and 257,

(Formula I)

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Met Ala Asn Lys Ala Val Asn Asp Phe Ile Leu Ala
1               5                  10
Met Asn Tyr Asp Lys Lys Lys Leu Leu Thr His Gln
            15                  20
Gly Glu Ser Ile Glu Asn Arg Phe Ile Lys Glu Gly
 25                  30                  35
Asn Gln Leu Pro Asp Glu Phe Val Val Ile Glu Arg
                40                  45
Lys Lys Arg Ser Leu Ser Thr Asn Thr Ser Asp Ile
      50                  55                  60
Ser Val Thr Ala Thr Asn Asp Ser Arg Leu Tyr Pro
                    65                  70
Gly Ala Leu Leu Val Val Asp Glu Thr Leu Leu Glu
            75                  80
Asn Asn Pro Thr Leu Leu Ala Val Asp Arg Ala Pro
 85                  90                  95
Met Thr Tyr Ser Ile Asp Leu Pro Gly Leu Ala Ser
                100                 105
Ser Asp Ser Phe Leu Gln Val Glu Asp Pro Ser Asn
    110                 115                 120
Ser Ser Val Arg Gly Ala Val Asn Asp Leu Leu Ala
                125                 130
Lys Trp His Gln Asp Tyr Gly Gln Val Asn Asn Val
            135                 140
Pro Ala Arg Met Gln Tyr Glu Lys Ile Thr Ala His
145                 150                 155
Ser Met Glu Gln Leu Lys Val Lys Phe Gly Ser Asp
                160                 165
Phe Glu Lys Thr Gly Asn Ser Leu Asp Ile Asp Phe
    170                 175                 180
Asn Ser Val His Ser Gly Glu Lys Gln Ile Gln Ile
                185                 190
Val Asn Phe Lys Gln Ile Tyr Tyr Thr Val Ser Val
            195                 200
Asp Ala Val Lys Asn Pro Gly Asp Val Phe Gln Asp
205                 210                 215
Thr Val Thr Val Glu Asp Leu Lys Gln Arg Gly Ile
                220                 225
Ser Ala Glu Arg Pro Leu Val Tyr Ile Ser Ser Val
    230                 235                 240
Ala Tyr Gly Arg Gln Val Tyr Leu Lys Leu Glu Thr
                245                 250
Thr Ser Lys Ser Asp Glu Val Glu Ala Ala Phe Glu
            255                 260
Ala Leu Ile Lys Gly Val Lys Val Ala Pro Gln Thr
265                 270                 275
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Glu Trp Lys Gln Ile Leu Asp Asn Thr Glu Val Lys
            280                 285
Ala Val Ile Leu Gly Gly Asp Pro Ser Ser Gly Ala
    290                 295                 300
Arg Val Val Thr Gly Lys Val Asp Met Val Glu Asp
                305                 310
Leu Ile Gln Glu Gly Ser Arg Phe Thr Ala Asp His
        315                 320
Pro Gly Leu Pro Ile Ser Tyr Thr Thr Ser Phe Leu
325                 330                 335
Arg Asp Asn Val Val Ala Thr Phe Gln Asn Ser Thr
            340                 345
Asp Tyr Val Glu Thr Lys Val Thr Ala Tyr Arg Asn
    350                 355                 360
Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val
                365                 370
Ala Gln Tyr Tyr Ile Thr Trp Asn Glu Leu Ser Tyr
        375                 380
Asp His Gln Gly Lys Glu Val Leu Thr Pro Lys Ala
385                 390                 395
Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His Phe
            400                 405
Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn
    410                 415                 420
Leu Ser Val Lys Ile Arg Glu Cys Thr Gly Leu Ala
                425                 430
Trp Glu Trp Trp Arg Thr Val Tyr Glu Lys Thr Asp
        435                 440
Leu Pro Leu Val Arg Lys Arg Thr Ile Ser Ile Trp
445                 450                 455
Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val
            460                 465
Glu Asn Asp
    470
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5. The modified pneumolysin according to claim 4, wherein a single amino acid substitution is made and the substituted amino acid is selected from the group consisting of proline or hydroxyproline for position 61; lysine, arginine or histidine for position 148 and leucine, glycine, alanine, isoleucine or valine for position 195.

6. The modified pneumolysin according to claim 3, wherein the substituted amino acids are selected from

the group consisting of serine, threonine, asparagine, glutamine, tyrosine or [cystine] cysteine for positions 33, 46 and 83; lysine, arginine or histidine for position 239 and leucine, glycine, alanine, isoleucine or valine for position 255.

7. Modified pneumolysin polypeptide pNVJ1.

8. Modified pneumolysin polypeptide pNVJ20

9. Modified pneumolysin polypeptide pNVJ22.

10. Modified pneumolysin polypeptide pNVJ45.

11. Modified pneumolysin polypeptide pNVJ56.

12. Modified pneumolysin polypeptide pNV103.

13. Modified pneumolysin polypeptide pNV207.

14. Modified pneumolysin polypeptide pNV111.

15. Modified pneumolysin polypeptide pNV211.

16. A recombinant nucleic acid molecule encoding a modified type 14 pneumolysin polypeptide wherein at least one amino acid in the region comprising amino acid residues 1 to 257 is substituted and wherein at least one of said amino acid substitutions results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

17. The recombinant nucleic acid molecule according to claim 16 comprising the following pneumolysin nucleic acid sequence:

ATGGCAAATA AAGCAGTAAA TGACTTTATA CTAGCTATGA 40
ATTACGATAA AAAGAAACTC TTGACCCATC AGGGAGAAAG 80

TATTGAAAAT CGTTTCATCA AAGAGGGTAA TCAGCTACCC 120
GATGAGTTTG TTGTTATCGA AAGAAAGAAG CGGAGCTTGT 160
CGACAAATAC AAGTGATATT TCTGTAACAG CTACCAACGA 200
CAGTCGCCTC TATCCTGGAG CACTTCTCGT AGTGGATGAG 240
ACCTTGTTAG AGAATAATCC CACTCTTCTT GCGGTCGATC 280
GTGCTCCGAT GACTTATAGT ATTGATTTGC CTGGTTTGGC 320
AAGTAGCGAT AGCTTTCTCC AAGTGGAAGA TCCCAGCAAT 360
TCAAGTGTTC GCGGAGCGGT AAACGATTTG TTGGCTAAGT 400
GGCATCAAGA TTATGGTCAG GTCAATAATG TCCCAGCTAG 440
AATGCAGTAT GAAAAAATCA CGGCTCACAG CATGGAACAA 480
CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGACAGGGA 520
ATTCTCTTGA TATTGATTTT AACTCTGTCC ATTCAGGCGA 560
AAAGCAGATT CAGATTGTTA ATTTTAAGCA GATTTATTAT 600
ACAGTCAGCG TAGACGCTGT TAAAAATCCA GGAGATGTGT 640
TTCAAGATAC TGTAACGGTA GAGGATTTAA AACAGAGAGG 680
AATTTCTGCA GAGCGTCCTT TGGTCTATAT TTCGAGTGTT 720
GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACGA 760
GTAAGAGTGA TGAAGTAGAG GCTGCTTTTG AAGCTTTGAT 800
AAAAGGAGTC AAGGTAGCTC CTCAGACAGA GTGGAAGCAG 840
ATTTTGGACA ATACAGAAGT GAAGGCGGTT ATTTTAGGGG 880
GCGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGGT 920
GGATATGGTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT 960
ACAGCAGATC ATCCAGGCTT GCCGATTTCC TATACAACTT 1000
CTTTTTTACG TGACAATGTA GTTGCGACCT TTCAAAATAG 1040
TACAGACTAT GTTGAGACTA AGGTTACAGC TTACAGAAAC 1080
GGAGATTTAC TGCTGGATCA TAGTGGTGCC TATGTTGCCC 1120
AATATTATAT TACTTGGAAT GAATTATCCT ATGATCATCA 1160
AGGTAAGGAA GTCTTGACTC CTAAGGCTTG GGACAGAAAT 1200
GGGCAGGATT TAACGGCTCA CTTTACCACT AGTATTCCTT 1240
TAAAAGGGAA TGTTCGTAAT CTCTCTGTCA AAATTAGAGA 1280
GTGTACCGGG CTTGCTTGGG AATGGTGGCG TACGGTTTAT 1320
GAAAAAACCG ATTTGCCACT AGTGCGTAAG CGGACGATTT 1360
CTATTTGGGG AACAACTCTC TATCCGCAGG TAGAAGATAA 1400
GGTAGAAAAT GAC 1413

and wherein said nucleic acid sequence comprises one or more of the nucleotide substitutions selected from the group consisting of:

A-50→G, G-54→T, T-181→C, A-196→T and T-302→C;

A-122→G, A-514→G, T-583→A and A-764→G;

A-187→T, T-380→A, A-382→C and T-443→A;

T-98→C, T-137→C, T-248→C, T-717→A and A-770→G;

T-134→C, A-305→G, A-566→G and T-583→G;

T-583→G;

T-583→A;

T-443→A;
and
T-181→C.

18. The recombinant nucleic acid molecule of claim 16 as contained in a vector such as a plasmid, cosmid, bacteriophage or yeast artificial chromosome.

19. A microorganism comprising the nucleic acid molecule of claim 16.

20. The microorganism according to claim 19, wherein the microorganism is selected from the group consisting of bacteria, yeast, mammalian or insect cells.

21. The microorganism according to claim 20, wherein the microorganism is *E. coli*.

22. The modified pneumolysin polypeptide of claim 1, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

23. The modified pneumolysin conjugate of claim 22, wherein the polysaccharide is from a bacteria selected from the group consisting of a Haemophilus influenzae type b; meningococcal group A, B or C; group B streptococcus types Ia, Ib, II, III, V or

VIII and pneumococcal.

24. A vaccine comprising at least one pneumolysin polypeptide of claim 1 and a pharmaceutically acceptable carrier.

25. The vaccine according to claim 24, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

26. The vaccine according to claim 25, wherein the polysaccharide is derived from a bacteria selected from the group consisting of Haemophilus influenzae type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus serotypes Ia, Ib, II, III, V, or VIII; or one or more of serotypes 1-23 of *S. pneumoniae*.

27. A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising the modified pneumolysin according to claim 1 in the presence of complement.

28. The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.

29. A method for immunization of mammals comprising administering the vaccine of claim 24 to said mammals.

30. A method for obtaining modified pneumolysin polypeptides having reduced hemolytic activity and

being suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

a) randomly mutating a nucleic acid molecule encoding for wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides and expressing the mutated nucleic acid molecules in host cells;

b) assaying the modified polypeptide expressed by the host cells for hemolytic activity;

c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.